1915

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THE INSOLUBLE BROMIDE VALUE OF OILS AND ITS DETERMINATION,

BY

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D. Sc. 1915.

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I, the undersigned, hereby declare that the work represented in this thesis was wholly carried out by myself, that the results therein are the true records of my investigation, and that the thesis was wholly composed by myself.

HISTORICAL AND OBJECT OF RESEARCH.

The determination of the "Hexabromide Value" or "Insoluble Bromide Value" of fatty oils has been the subject of several researches since the original method was published by Hehner & Mitchell, (Analyst 1898, XXIII, 313). The investigations have usually been confined to the determination of the amount of precipitate obtained by brominating solutions of the oils or of their fatty acids, the solvent for the oil in most cases being ether. As the results published in the various papers however, sometimes varied within wide limits, it was deemed necessary that further investigation would have to be made, if this so - called "Value" was to serve any useful purpose. The primary object of this research was, therefore, to examine the various methods published, and if possible to select the best one as a standard method, failing which, to attempt to modify the process so as to obtain a simple yet reliable analytical process. Later, however, the field of work was extended to include an examination as to the nature of the precipitates obtained, as very little previous work seems to have been done on the subject.

The following paragraphs briefly describe the methods published to date:-

HEHNER & MITCHELL. (Loc.Cit.) 1 to 2 grams of the oil dissolved in 40 c.c. of ether to which a few c.c. of glacial acetic acid have been added, are cooled to 5° C. in a corked flask, and bromine added drop by drop until the brown colouration remains permanent. The solution is allowed to stand for three hours, is then filtered through an asbestos filter, and the precipitate washed successively with 5 c.c./

5 c.c. portions of chilled glacial acetic acid, alcohol, and ether. The residue is finally dried to constant weight in a water oven. In the case of fish oils, the filtering medium recommended is chamois leather, owing to the fineness of the precipitate. The use of acetic acid is for the purpose of obtaining the precipitate in a granular condition.

WALKER & WARBURTON. (Analyst 1902, 27, 237) use the previous method and publish a list of constants obtained from several varieties of oil, giving comparative results of the figures obtained by brominating the glycerides and fatty acids respectively. Certain discrepancies in the results are not, however, explained.

PROCTOR. (Journ. Soc. Chem. Ind. 1906, 25, 198) confines himself to an examination of the marine animal oils. He mentions the difficulty experienced in filtering the product obtained by brominating the glycerides dissolved in ether. He recommends the use of carbon tetra - chloride, followed by a further addition of carbon tetra - chloride containing .75 percent of phenol (for the purpose of getting rid of excess of bromine), and finally the addition of absolute alcohol. The precipitate after transferring to a weighed filter paper is washed with absolute alcohol and dried to constant weight.

BULL & JOHANNSEN. (Chem. Zeit. 1909, 73) recommended the following procedure:- 1 gram of the fatty acids is dissolved in 5 c.c. of glacial acetic acid and 25 c.c. of ether. .5 c.c. of bromine is now slowly added with cooling and shaking. After standing, the precipitate is filtered off, washed thrice with 5 c.c. of the ether - acid mixture, and twice with ether. The authors state that the results/

results vary with the proportion of the reagents used, and also with the amount of bromine added.

EIBNER & MUGGENTHALER. (Farbenzeitung 1912, 131, 175, 235, 356, 411, 466, 523, 582, 641) recommend the use of the fatty acids obtained from three successive portions of 3.5 grams of oil, saponified separately, and the whole dissolved in 180 c.c. of water. The acids are liberated from the soap with sulphuric acid and extracted with 140 c.c. of ether. The ethereal solution is allowed to stand overnight over 70 grams of dehydrated potassium sulphate, the ether filtered, and the potassium sulphate washed with dry ether. The ether is next evaporated, the last portions in a current of hydrogen, and the fatty acids dried in vacuo, over concentrated sulphuric acid. A solution of the acids of approximately 10 per cent strength is made in dry ether, and 20 c.c. portions brominated at -10° C., using 1 c.c. of bromine. After standing for two hours at -10° C. the mother liquor is decanted through a weighed Daniel tube, and the precipitate is shaken up with 5 c.c. of ether at -10° C., again decanted, and again shaken up with 5 c.c. of cooled ether. The flask is now washed out with three successive portions of 5 c.c. of ether at -10° C., and the tube drained, and dried at 80° - 85° C.

SUTCLIFFE. (Analyst 1914, 39, 28) brominates the glycerides dissolved in ether and glacial acetic acid, 1 gram of the oil being dissolved in 40 c.c. of ether and 5 c.c. of glacial acetic acid, and cooled in running water. The brominated solution is allowed to stand in water over-night, then decanted through a Gooch crucible, washed thrice by decantation with 10 c.c. of cooled ether, and finally twice in the crucible. The precipitate is dried for three hours in the water oven, cooled, and weighed.

This paper was published subsequent to the present research being commenced.

EXPERIMENTAL.

PART I.

RESULTS OBTAINED BY TRIAL OF PUBLISHED METHODS.

The original method of Hehner & Mitchell (loc. cit.) was first tried. The authors do not give any definite proportion of glacial acetic acid to be used in the solvent, but simply state "a few c.c." Seven determinations were made in four of which the oil was dissolved in 35 c.c. of ether and 5 c.c. of glacial acetic acid, and in three of which the oil was dissolved in 40 c.c. of ether without acetic acid. A sample of refined linseed oil was used. The following results were obtained:-

A. Oil dissolved in 35 c.c. Ether and 5 c.c. glacial acetic acid.

Grams Oil.	Grams of Bromides.	Per cent of Bromides.
1.5000	0.5539	36.93%
2.2850	0.9065	39.66%
2.2525	0.9105	40.41%
2.2700	0.9230	40.50%

B. Oil dissolved in 40 c.c. ether.

2.3370	1.0275	44.74%
2.1750	0.8970	41.34%
2.1520	0.9690	45.03%

Considerable difficulty was experienced in the first washing of the precipitates, where the wash liquid was glacial acetic acid, owing to the acid crystallizing, otherwise the method worked well. In the absence of acetic acid in the solvent, filtration of the precipitate was slow, and difficulty was experienced in washing, thus bearing out the authors'/

authors' statement that the presence of the acid is necessary to obtain a granular precipitate. The results obtained in absence of the acid also, were far from concordant. The results obtained in table A. above show that apparently, concordant results can be obtained by this method, provided that approximately the same weight of oil is taken for each determination, and that the conditions of working are the same in all cases. The authors however, leave three points open for variation in procedure, viz:- (1). No definite weight of oil is used. They state "1 to 2 grams". (2). The proportions of ether and glacial acetic acid to be used in the solvent for the oil are not definitely stated. (3). Bromine is to be added "till the brown colour remains permanent". This is found to be a very indefinite point in practice, and I have found that precipitation is not complete even at this stage, but that bromine must be added until the supernatant liquid is red. This has since been confirmed by Sutcliffe. (loc. cit.)

It is further to be noted that although concordance in results was obtained in the first series of experiments, further determinations made, using the same sample of oil and the same reagents as in the first series, gave results which varied from those previously obtained, the figures obtained varying from 37% to 42%, the weight of oil used being approximately the same as that used in the last three determinations in table A.

A further series of experiments was made with the object of determining whether the proportion of glacial acetic acid used in the ether - acid solvent, made any appreciable difference in the results obtained. The following results/

results were obtained:-

c.c. Glacial Acetic Acid.	Per Cent Bromides.	
	Sample A.	Sample B.
2.	------	38.75
5.	39.85	37.85
10.	35.50	33.65
15.	30.40	-----

This result has been confirmed by Sutcliffe. (loc. cit.)

The results obtained by an examination of this method show conclusively that, as it stands, the method of working as recommended by the authors, requires considerable alteration, more especially in regard to the use of a definite weight of oil for examination, the necessity for a fixed proportion of glacial acetic acid in the solvent mixture, and the addition of bromine until the supernatant liquid is red.

The use of chamois leather as a filtering medium for the precipitate obtained by brominating solutions of the marine animal oils was also found to be quite useless, thus confirming the observations of Proctor (loc. cit.) in this respect.

The second published method attempted was that of Proctor, (loc. cit.). As this method was specially recommended for the examination of marine animal oils, a sample of cod liver oil was used. It was considered doubtful if the method would be a satisfactory one, principally on account of absolute alcohol being used as the wash liquid and also as the precipitant for the bromide dissolved in carbon tetra - chloride. To determine whether the actual strength of the alcohol used made any difference, two experiments were conducted side by side, in one of which alcohol of 98.6 per cent strength by weight was used, and in the other, alcohol of 92.1 per cent by weight.

The mean of the results obtained showed 50.00 per cent of precipitate when 98.6 per cent alcohol was used and 45.75 per cent where 92.1 per cent alcohol was used. As alcohol is so hygroscopic and liable to lose strength quickly, it is obvious that variations are liable to arise on this account.

The precipitates obtained by this method were also found exceedingly difficult to filter, more especially those where the alcohol approximated to absolute, several hours being required for the filtration alone. The precipitates, however, dried a good colour.

The method of Sutcliffe (loc. cit.) was tried immediately after it was published, although the study of the problem from the point of view of the bromination of the glycerides themselves had been given up some time previously.

Three series of experiments were carried out in all, in each series of four determinations the published method being closely adhered to. The results were as follows:-

Series 1. Per-centage of Bromides.	Series 2. Per-centage of Bromides.	Series 3. Per-centage of Bromides.
41.20	45.10	42.80
45.10	47.90	45.80
47.70	48.50	46.00
41.40	48.30	45.10

It is apparent from the above figures that this method is no better as regards constancy of results obtained than those methods previously tried. Again, exception may be taken to the fact that variation in temperature is liable to arise, as the temperature of tap water, which the author of the paper recommends, is liable to seasonable variations.

The two remaining methods, viz:- those of Bull &/

& Johannsen, and Eibner & Muggenthaler, (loc. cit.) recommend the bromination of the fatty acids liberated from the oils as distinguished from the bromination of the glycerides themselves, which is the procedure adopted in the methods of Hehner & Mitchell, Proctor, and Sutcliffe.

Bull & Johannsen's method was found to give good results in experiments carried out in series, but consecutive series, using the same sample of oil, gave varying results. This is probably due to the fact that oxidation of the unsaturated fatty acids takes place to some extent during the preparation of the sample, unless precautions are taken to carefully exclude oxygen during the process of drying, a procedure which in practice one is not always able to adopt. The effect of oxidation is shown in the results obtained in the two following series of experiments, the same sample of oil being used in each case:-

Per centage of Bromides.	
Series A.	Series B.
26.70	22.60
26.90	23.35
25.90	22.40
	23.15

As an analytical process, the method of Eibner & Muggenthaler was found to be much too complicated, and unless very carefully carried out, losses were liable to occur during the preparation of the fatty acids, firstly, owing to the incomplete washing of the potassium sulphate over which the ethereal solution of the acids is dried, and secondly, as mentioned in the method of Bull & Johannsen, owing to oxidation of the acids during drying, unless great precautions are taken to prevent this. Again great difficulty was experienced in maintaining a constant/

constant temperature of -10° C. at which temperature the determinations are made, and lastly it was not found possible to wash the precipitated bromides free from bromine and the bromides of the lower fatty acids with the volume of wash liquid recommended by the authors, with the result that the precipitates dried brown and sticky. The whole process too, is lengthy and cumbersome, the authors' estimate of two days being the minimum of time required for the determination.

Only two results were obtained by the use of this method and these varied widely, viz:- 38.1 per cent, and 44.4 per cent respectively.

PART 2.

RESULTS OF MODIFICATIONS OF PUBLISHED METHODS.

As none of the published methods were found to work with complete satisfaction, attempts were made to find whether modification of any of the above methods would give reliable results. Of the methods previously mentioned, those of Hehner & Mitchell, and Bull & Johannsen seemed the most likely to yield good results by slight variations in the published methods.

Modifications of Hehner & Mitchell's method were first attempted. It was thought that better results might be obtained if the oil was dissolved in some solvent other than ether. The following trials were made:-

A. Petroleum Spirit. The oil was dissolved in 30 c.c. of Petroleum spirits and 1 c.c. of bromine added drop by drop. The bromo-glycerides of linoleic and linolenic acid should be precipitated. After standing, the supernatant liquid was decanted and the precipitate washed several times by decantation with cooled ether containing 5 per cent of glacial/

glacial acetic acid, and finally filtered through a Gooch crucible. This treatment should remove the tetra - bromo - glyceride. Great difficulty, however, was experienced in washing the precipitate, and filtration was slow. On drying, the precipitate became brown and sticky. The following results were obtained:-

Oil Taken.	Per-centage of Bromides.
2.3075 Grams.	37.70%
2.3590 "	35.93%

B. Alcohol - Ether. The oil was dissolved in 30 c.c. of a mixture of equal volumes of ether and rectified spirits, and 1 c.c. of bromine slowly added to the cooled mixture. After standing, the brown liquid was decanted, the precipitate was washed with cooled ether, and filtered through a Gooch crucible. The product was difficult to filter and wash free from bromine, and dried brown and sticky.

C. Alcohol. The same difficulty in washing and filtering was experienced as in the two preceding methods, and results were found to vary according to the strength of the alcohol.

D. Chloroform. All the bromo - derivatives were found to be soluble in this solvent, even on cooling to -5° C. and standing for four hours.

It is clear from this series of experiments that no advantage is to be gained by the use of any of the other solvents tried, and that ether, containing a fixed proportion of glacial acetic acid (the necessity for such a fixed proportion being evident from the result of experiments shown on page 6) appears to be the best solvent medium in which to carry out the precipitation.

Investigation was next made to determine whether any variation in the method of precipitation or of washing the precipitate would give better results.

Series A. Nos. 1 to 3. The oil was dissolved in 25 c.c. of ether, cooled, and 1 c.c. of bromine dissolved in 15 c.c. of ether added. Nos. 4 to 6. The oil was dissolved in 20 c.c. of ether and and 5 c.c. of glacial acetic acid, cooled, and 1 c.c. of bromine dissolved in 15 c.c. of ether added. The flasks containing the precipitates were allowed to stand in ice water for several hours, and after decantation of the supernatant liquid, the precipitates were filtered and washed, as follows:- First, with 10 c.c. of cooled ether containing 5 per cent of glacial acetic acid, followed successively by 10 c.c. and 5 c.c. of cooled ether, and 5 c.c. of cooled rectified spirits (twice). The precipitates dried to a good colour, but in quantity showed variations of, in some cases, 3 per cent.

No.	Grams of Oil.	Grams of Bromide Found.	Per-centage of Bromides.
1.	1.4835	0.8390	56.55%
2.	1.4630	0.8335	56.97%
3.	1.4240	0.7595	53.34%
4.	1.5925	0.8240	51.74%
5.	1.6530	0.8685	53.14%
6.	1.6195	0.8675	53.56%

Series B. Six lots of oil were dissolved, each in 35 c.c. of ether, and 5 c.c. of glacial acetic acid, cooled in ice water, and bromine added drop by drop till in excess. After standing in ice water for several hours, the supernatant liquid was decanted and the precipitates filtered and washed as in the previous series. The variation between individual/

individual determinations was as great as in Series A., while the actual percentage of precipitate obtained was much less.

No.	Grams of Oil.	Grams of Bromide Found.	Per-centage of Bromides.
1.	1.4045	0.5820	41.43%
2.	1.3930	0.5430	38.99%
3.	1.4370	0.5645	39.34%
4.	1.4225	0.6110	42.94%
5.	1.3930	0.6175	44.33%
6.	1.4680	0.6110	41.62%

Series C. Six lots of oil were dissolved in 20 c.c. of ether. To Nos. 1 to 3 was added 15 c.c., and to Nos. 4 to 6, 10 c.c. of a mixture containing:- Ether - 180 c.c.; Bromine - 10 c.c. Glacial acetic acid - 10 c.c. The filtration and washing process was as in the previous two series. The results obtained by this method showed that using approximately the same weight of oil, greater constancy resulted than in the previous two series, and led to the belief that if the weight of oil under examination was kept constant, variations in the individual determinations might be reduced, and so allow of a reliable analytical process being devised.

No.	Grams of Oil.	Grams of Bromide Found.	Per-centage of Bromides.
1.	1.4605	0.7750	53.10%
2.	1.4610	0.7760	53.11%
3.	1.4925	0.7900	52.93%
4.	0.7370	0.3090	41.92%
5.	0.7610	0.3285	43.03%
6.	0.7410	0.3100	41.83%

It was found on further examination that the ether - bromine - acetic acid mixture was unstable, so the following slight alteration in the procedure was made. The oil was dissolved in 18 c.c. of ether and 2 c.c. of glacial acetic/

acetic acid, cooled, and a cooled, freshly prepared mixture of 19 c.c. of ether and 1 c.c. of bromine was slowly added. The precipitates in every case were filtered and washed according to the method described in Series A.

Series D. - Effect of Time of Standing on Percentage of Bromides Obtained.

Two gram lots of oil were weighed into Erlenmeyer flasks, treated as in Series C. and the brominated solutions allowed to stand in ice water for varying lengths of time.

Two points were apparent from the figures obtained, viz:- that greater constancy in the results can be obtained by the use of a definite weight of oil than if any arbitrary amount is used, and also that variations of at least 1 per cent in consecutive results were obtained unless the brominated solutions were allowed to stand for at least four hours, and also that on standing for still longer periods, slight variations were observed.

Time, in Hours.	Grams of Bromide Found.	Per-centage of Bromides.
1.	0.6520	32.60%
1.	0.8340	41.70%
2.	0.9390	46.95%
2.	0.9130	45.65%
4.	0.9650	48.25%
4.	0.9730	48.65%
6.	0.9480	47.40%
6.	0.9400	47.00%
10.	0.9580	47.90%
10.	0.9650	48.25%
18.	0.9425	47.12%

Series E. - Effect of Quantity of Oil Taken on Percentage of Bromides Obtained.

Quantities of oil varying from 0.5 to 2.5 grams were weighed into flasks, treated as in Series C. and allowed to stand in/

in ice water for four hours. An examination of the results obtained at once showed the necessity for the use of a fixed quantity of oil. The smaller the amount of oil taken, the lower was the result, and the greater was the variation in consecutive experiments. Two gram lots of oil were found to give less variation than smaller quantities, while the precipitate was readily freed from bromine and the other products of the reaction, with the volumes of wash liquid recommended in Series A. Larger weights of oil, although giving higher values, were not so easily washed.

Grams of Oil.	Grams of Bromide Found.	Percentage of Bromides.
0.5	0.1410	28.20%
0.5	0.1675	33.50%
1.0	0.3670	36.70%
1.0	0.3490	34.90%
1.5	0.6355	42.37%
1.5	0.6650	44.33%
2.0	0.9785	48.92%
2.0	0.9720	48.60%
2.5	1.3170	52.68%

When this point in the investigation was reached, the author considered that whatever the nature of the precipitate was, the problem of obtaining constant results was likely to be solved by this method. In order to prove this conclusion, several samples of linseed oil were examined. In each case 2 grams of the oil were used, brominated according to the method recommended, and the brominated solutions allowed to stand in ice water for four hours, filtered, washed, and dried.

No. 1. Refined Linseed. Iodine Value.	No. 2. Raw Linseed. Iodine Value.	No. 3. Boiled Linseed. Iodine Value.	No. 4. Boiled Linseed Iodine Value.
173.5	171.7	168.7	184.9
34.63	35.40	30.00	43.85
35.25	35.70	30.70	43.90
35.78	36.15	31.23	44.15
35.15	36.25	31.40	45.10
36.30	36.65	32.40	45.15
36.75	36.75	32.55	45.45
37.25	37.67	32.63	45.65
37.03	37.80	32.70	45.85
37.60	38.25	32.75	46.45
38.18	38.30	32.75	46.95
38.68	38.40	32.80	.....
38.75	38.60	35.65	.....
38.60	39.00	.....	.....
38.97	40.65	.....	.....
38.30	.....	.....	.....
41.22	.....	.....	.....
Average 37.40	37.54	32.29	45.25

These results show that conducting the investigation over a prolonged series of experiments, very considerable differences arise between the maximum and minimum values obtained. The determinations were conducted in series of three or four at one time, and frequently these series showed concordance between individual determinations in the series, but sometimes differed to a considerable extent from the results obtained in the other series where the same oil was under examination.

The following table shows the figures obtained in each sub - series, with oil No. 1 under examination:-

	Percentage of Bromides.		
Sub - series No. 1.	Sub - series No. 2.	Sub - series No. 3.	Sub - series No. 4.
38.75	34.63	37.03	41.22
38.18	35.25	36.75	38.97
38.68	35.78	37.60	38.30
37.25	36.20	35.15	38.60

It should be noted, however, that the majority of the results lay within 1 per cent on either side of the average of the whole series. It is obvious, however, that it would be impracticable to carry out such a large number of determinations where routine examination of a large number of samples was to be carried out. Although as far as could be determined, the procedure adopted was in every case identical, it appears as if certain personal factors entered into the problem which are difficult to eliminate. If such is the case, it seems quite likely that two investigators by constant practice, each using the same method, might obtain concordant results, which would possibly be quite different from each other as a whole. As bearing out the fact that slight differences in procedure make comparatively large differences in results, if an examination is made of the results obtained in Series A. and B., (pages 11 and 12) where the oil under examination was the same in each series, it is to be noted that the difference in procedure adopted gave results which varied very considerably, taking the average of results obtained in each series.

A method somewhat on the lines adopted by Proctor, (loc. cit.) was next tried. Two gram lots of oil were dissolved in 20 c.c. of chloroform - ether mixtures containing various proportions of the two solvents, 2 c.c. of glacial acetic acid added, and after cooling, brominated with 20 c.c. of ether containing 1 c.c. of bromine. The following results were obtained:-

Percentage Bromides using 20 c.c. of Chloroform.	Percentage Bromides using 10 c.c. of Chloroform & 10 c.c. of Ether.	Percentage Bromides using 5 c.c. of Chloroform and 15 c.c. of Ether.	Percentage Bromides using 20 c.c. of Ether.
25.65	28.20	35.90	35.20
24.75	29.45	35.10	35.65
24.85	28.55	35.90	36.55
25.08	28.73	35.63	35.80

Here again there was apparent uniformity in the results which, however, disappeared on carrying out a larger series of experiments, for example, using 10 c.c. of chloroform and 10 c.c. of ether, results were obtained varying from 28.2 per cent to 32.45 per cent.

This concluded for the time being the investigation from the point of view of direct bromination of the glycerides themselves. More recently, however, the effect of using pure methylated ether, (Sp. Gr. .917) in place of commercial methylated ether, (Sp. Gr. .930), and without the use of alcohol in the washing process was tried, as it was thought that the presence of alcohol might be the cause of the variation in results. The results obtained, however, were no better than in the previous trials made, as is shown by the following results obtained by use of a sample of Plate linseed oil.

Percentage of Bromides.
40.10%
37.85%
38.90%
40.20%
38.00%

Modifications of the existing methods based on the direct bromination of the glycerides having proved unsatisfactory, an attempt was next made to ascertain if Bull & Johannsen's method/

method could be so far modified as to give concordant results.

The first trial was carried out as follows:- Four one gram lots of linseed oil were saponified with alcoholic potash, evaporated to dryness, and the soap dissolved in water. The fatty acids were liberated with sulphuric acid and each lot extracted with 30 c.c. in all of ether, 2 c.c. of glacial acetic acid was added, the whole cooled in ice water, bromine added drop by drop till in excess, and the mixture allowed to stand for some time in ice water. The ether was decanted through weighed papers, 10 c.c. of cooled ether added and decanted, the same operation repeated three times with 5 c.c. of cooled ether, and the precipitate finally transferred to the paper and washed twice with 5 c.c. of cooled ether, dried, and weighed.

Percentage of Bromides.
20.90%
21.90%
19.70%
19.40%

As the results obtained by this procedure were not constant, the following modification was used:- 5 grams of the oil were saponified with alcoholic potash, the soap was dried, dissolved in 100 c.c. of water, and the fatty acids liberated with sulphuric acid. The whole was cooled under the tap, about 30 c.c. of ether added, the flask rotated to allow of the mixing of the ethereal with the aqueous layer, and corked for some time. The contents of the flask were then transferred to a separator, and the aqueous layer separated. The aqueous layer was re - extracted twice with 20 c.c. portions of ether, and the whole ethereal extract (filtered if/

if necessary through a dry filter paper) made up to 100 c.c. Four lots of 20 c.c. (each representing the acids from 1 gram of oil) were pipetted into flasks, 2 c.c. of glacial acetic acid added to each, the flasks cooled in ice water, and the solutions brominated, and allowed to stand in ice water. The brown mother - liquors were decanted, and the precipitates washed thrice by decantation with 5 c.c. portions of cooled ether, and twice, after transference to weighed papers, with the same volume, dried, and weighed. The results were as follows:-

Series 1.	Series 2.
Percentage of Bromides.	Percentage of Bromides.
27.50%	28.05%
28.30%	27.90%
28.10%	27.70%
28.25%	28.35%

This method appeared satisfactory, and was found to give concordant results with consecutive series of determinations as is shown by the results obtained in the table above.

Effect of Time on Percentage of Bromides Obtained. Experiments were conducted to ascertain the minimum time which the brominated solutions should stand to allow of complete precipitation taking place. The results showed that after two hours no further precipitate appeared on allowing to stand for a further period of two hours. A further secondary precipitate appeared in the case of the vegetable oils after several hours, accompanied by the separation of a heavy liquid. This precipitate on filtration, was found to consist of very impure bromide which dried brown and sticky, and was rejected. The following table shows the effect of time:-

Time, in minutes.	Percentage of Bromides.	Average of determinations over same period.	Further percentage precipitated on standing to make total time 2 hours.	Total percentage of Bromides.
15 15	31.50% 29.60%	30.55%	4.20%	34.75%
30 30	33.30% 32.40%	32.85%	2.35%	35.20%
60 60	34.25% 34.00%	34.12%	1.15%	35.27%
120 120	34.75% 34.50%	34.62%	nil.	34.62%

Effect of Concentration on Percentage of Bromides Obtained.

It was found that the percentage of precipitate obtained varied according to the concentration of the solutions of acids brominated, - the greater the concentration, the larger the percentage. Twenty c.c. portions of solutions of less than 5 per cent concentration gave results which were liable to variation, while in twenty c.c. portions of solutions of greater concentration than 5 per cent, difficulty was experienced in cases where large quantities of bromides were precipitated, in washing the precipitates free from bromine and other products of the reaction. In the latter case also the precipitate was found to be less pure than where a 5 per cent solution was employed. (See sections dealing with nature of precipitates.) Even a concentration of 5 per cent however, was found in certain instances to yield such large precipitates that washing according to the original method with five successive portions of 5 c.c. of cooled ether in the flasks and on the paper was insufficient. The following method, however, overcame this difficulty:- After standing/

standing for two hours, the brown liquor was decanted from the precipitate as far as possible, and the residue transferred to a centrifuge tube, centrifuged, and the liquid poured off. 5 c.c. of cooled ether were added to the flask, the sides of the flask cleaned with the aid of a rubber - tipped rod, and the liquid transferred to the centrifuge tube. The precipitate was thoroughly mixed with the wash liquid by means of a pointed glass rod, the whole centrifuged, and the liquid poured off. This was repeated, then 5 c.c. of cooled ether added to the tube, the precipitate and ether mixed, and the whole transferred to a weighed paper, the tube washed out with 5 c.c. of cooled ether, and the precipitate finally washed on the paper with 5 c.c. of cooled ether. In this way, the precipitates were washed quite free from bromine and this method of washing was adopted in all following determinations.

Effect of Volume of 5 percent Solution used on Percentage of Bromides Obtained. Series of experiments were conducted to ascertain if the percentage of precipitate obtained depended on the volume of 5 per cent solution brominated. The results obtained by an examination of several samples of linseed oil showed that the amount of precipitate varied considerably with the volume, as is shown by the following table:-

c.c. of solution.	Equal to Grams of Oil.	Sample A.	Sample B.	Sample C.	Sample D.
10	0.5	30.60	22.40	23.90	21.20
20	1.0	36.25	29.85	30.20	25.75
30	1.5	41.47	34.00	33.86	28.25
40	2.0	42.45	36.15	34.88	29.40

With oils yielding low percentages of precipitate, naturally the use of more concentrated solutions or larger/

larger volumes of the 5 percent solution, would yield higher values and would probably give greater concordance between consecutive results, but if the method is to be of any real value, the procedure adopted must remain constant.

One conclusion arrived at by a study of the figures obtained in the last three series of experiments was that in all probability the problem was one in which the solubility of the bromide in ether had to be taken into consideration. To ascertain if this was the case or not, the mother - liquors were concentrated to approximately half their original volume, cooled, and allowed to stand in ice water for two hours. In all cases further precipitates were obtained, varying to some extent owing to the concentration being slightly different in each case, but at any rate proving that the conclusion arrived at was correct.

The results of a series of experiments to prove this conclusion are given below:-

c.c. of 5 per cent solution.	Equal to Grams of Oil.	Percentage of Bromide. First crop.	Second crop.	Third crop.	Total
10	0.5	21.20	5.40	3.20	30.00
20	1.0	25.75	4.65	1.40	31.80
30	1.5	28.26	4.60	.60	33.46
40	2.0	29.40	4.30	.40	34.10

In order to further prove this point, samples of pure linoleic and linolenic acids were obtained, and a series of experiments conducted, various mixtures of ethereal solutions of the two being used, with the following results:-

LINOLEIC ACID.

Grams of Acid.	c.c. of glacial acetic acid.	Total volume of solution brominated.	Grams of Bromide.
.2136	nil.	10 c.c.	0.036
.2136	nil.	20 c.c.	0.028
.2136	2.0	10 c.c.	0.027
.2136	2.0	20 c.c.	0.023

LINOLENIC ACID.

Grams of Acid.	c.c. of glacial acetic acid.	Total volume of solution brominated.	Grams of Bromide.	Percentage of actual weight required by theory.
.2518	nil.	10 c.c.	0.098	14.40%
.2518	2.0	10 c.c.	0.065	9.54%
.2518	2.0	20 c.c.	0.058	8.50%
.5036	nil.	10 c.c.	0.256	18.80%
.5036	2.0	20 c.c.	0.198	14.59%

LINOLEIC and LINOLENIC ACID.

Grams of Linoleic Acid.	Grams of Linolenic Acid.	c.c. of glacial acetic acid.	Total volume of solution brominated.	Grams of Bromides.	Percentage actual weight required by theory.
.2136	.2518	nil.	10 c.c.	0.169	24.82%
.2136	.2518	2.0	10 c.c.	0.146	21.44%
.2136	.5036	nil.	10 c.c.	0.340	24.96%
.2136	.5036	2.0	10 c.c.	0.293	21.51%
.2136	.2518	nil.	20 c.c.	0.155	22.76%
.2136	.2518	2.0	20 c.c.	0.131	19.23%
.2136	.5036	nil.	20 c.c.	0.326	23.93%
.2136	.5036	2.0	20 c.c.	0.283	20.77%

A study of these results shows that standard conditions are necessary both as regards concentration of the solution of acids to be brominated, and volume of wash liquid to be used, as it is obvious that the chief substance producing the precipitate, viz:- linolenic acid hexabromide is a soluble one, and results will vary according to the method adopted. The effect of acetic acid is also very noticeable, the results being about 16 per cent lower than where no acetic acid is present. Its use, however, is to be preferred, since in its presence the product has been found to approximate more closely to linolenic hexabromide, (see sections dealing with nature of precipitates). The use of 10 per cent by volume of glacial acetic acid in the solution to be brominated was found to be quite sufficient. It is also to be noted that linoleic acid produces a slight precipitate even in a 5 per cent ethereal solution of the acid containing 10 per cent by volume of acetic acid.

The solubility of the hexabromide in ether is not, however, so great as it seems by an examination of the above results. The following figures were obtained by a solubility test carried out on the moist precipitate obtained by brominating pure linolenic acid, and after washing free from bromine, immersed in pure ether, the whole boiled for one hour, and then cooled:-

	Grams per 100 c.c.	
Solubility at:-	In Pure Ether.	In Ether containing 10 percent of glacial acetic acid.
0° C.	.245	.361
15.5° C.	.335	.482

It thus appears that complete precipitation does not take place when the acids are brominated, but that this is not altogether due to the hexabromide being soluble, but is chiefly due to some other cause the nature of which the author has been unable to discover.

Effect of Purity of Ether on Percentage of Bromides Obtained.

A series of experiments was made using ordinary methylated ether, (Sp. Gr. 730) compared with anhydrous methylated ether, (Sp. Gr. 917). It was found that in every case lower results were obtained with commercial ether, but that when this was purified by standing over solid caustic soda and subsequent distillation, the results were the same as when pure ether was used, thus proving that the presence of alcohol accounted for the difference in results. The presence of water did not appear to affect the results. The ether was thereafter purified by treatment with solid caustic soda, re-distilled, and dried over anhydrous copper sulphate or potassium carbonate.

Percentage of Bromides, using commercial ether.	Percentage of Bromides, using pure ether.
29.80%	32.50%
29.05%	32.70%
30.00%	32.40%
29.15%	32.80%

Determination of the Insoluble Bromides in Marine Animal Oils.

It was found on trial that marine animal oils also gave satisfactory results using the same method as that adopted for vegetable oils. The precipitates obtained, however, were more finely divided, and it was found advisable to filter through Swedish filter paper. The solubility factor as far as could be ascertained was not so great as in the case of vegetable oils, and a precipitate appeared whenever bromine/

bromine was added. In the case of the vegetable oils the precipitate did not appear till practically all the bromine had been added. The results too, where varying volumes of a 5 per cent solution of the acids were employed did not vary within such wide limits (compare with results on page 22). The following are the results obtained from a sample of whale oil:-

		Percentage of Bromides.			
Grams of oil.	Volume of 5 per cent solution used.	First Crop.	Second Crop.	Third Crop.	Total.
0.5	10	22.20	3.20	1.12	26.52
1.0	20	23.70	2.85	.90	27.45
1.5	30	24.33	2.13	.60	27.06
2.0	40	24.65	1.90	.40	26.95

The following table gives a detailed list of oils examined by the method recommended. In every case the result for each oil was the average of eight determinations carried out in two series of four determinations each. In no case did the results differ by more than 1 per cent in the whole series of eight determinations. There appears to be no exact relationship between the percentage of precipitate and the iodine value, but this is not to be expected as the precipitates vary to some extent in composition according to the proportions of linoleic and linolenic acid present in the oil. (vide infra).

Vegetable Oils.

	Iodine value.	Bromides, per cent.
Raw Linseed Oils.		
Refined (origin unknown).	182.5	34.00
Raw. " "	182.0	33.65
Matured Baltic.	197.5	37.65
Baltic - Sample A.	196.0	36.10
" B.	184.7	32.60
Plate - Sample A.	176.5	33.30
" B.	175.6	32.60

	Iodine value.	Bromides, per cent.
Boiled Linseed Oils.		
Baltic.	170.5	25.95
Plate.	165.5	27.75
Plate - Sample A.	184.9	33.90
" B.	180.2	30.20
" C.	176.3	27.10
" D.	172.5	26.65
Soya Bean.	141.8	4.10
Rape - Sample A.	108.0	2.35
Rape ? - B.	122.8	5.80
Walnut.	148.2	3.00
Tung.	164.2	nil.

Marine Animal Oils.

	Iodine value.	Bromides, per cent.
Cod liver.	172.2	35.20
Whale.	149.3	21.70
Brown whale.	139.6	25.80
Menhaden,	170.8	51.70
Shark liver.	119.1	17.70
Seal?	166.9	29.12
Sperm.	81.2	1.70

Part 3.

NATURE AND BROMINE CONTENT OF THE PRECIPITATES.

Hehner & Mitchell in their paper stated that the precipitates obtained by them contained on an average about 56 per cent of bromine, whereas theory required 62.26 per cent for the hexa-bromo-glyceride of linolenic acid. An ultimate analysis yielded the following percentage composition:-

Carbon.	33.29
Hydrogen.	5.48
Oxygen.	4.49
Bromine.	56.74

and those authors assigned the following formula to the precipitate - $C_3H_5\begin{cases}C_{18}H_{29}O_2Br_6\\C_{18}H_{29}O_2Br_6\\C_{18}H_{33}O_2Br_2\end{cases}$ which by theory should give an analysis of:-

Carbon.	34.27
Hydrogen.	4.81
Oxygen.	4.81
Bromine.	56.11

The following methods for the estimation of the bromine were tried in the present investigation:-

Method A. A weighed quantity of the bromides (usually .25 grams) was intimately mixed with 2 grams of soda lime, and heated over a low rose - burner in a porcelain crucible for half an hour, the temperature being gradually raised until the crucible was heated by the full flame of the rose - burner. The sintered mass was extracted with boiling water, filtered, and washed, the filtrate cooled and acidified with dilute nitric acid, excess of $^N/_{10}$ silver nitrate added, and the excess back-titrated with $^N/_{10}$ potassium thio-cyanate, using ferric alum as indicator. A blank determination with the soda lime was carried out at the same time.

Method B. A weighed quantity of the bromides was boiled under a reflux condenser with excess of alcoholic caustic/

caustic potash, and after complete saponification the alcohol was evaporated, the soap dissolved in water, acidified with dilute nitric acid and titrated as in the previous method. It was found that at least six hours' boiling was necessary to completely decompose the bromide using .25 gram of bromide and 10 c.c. of $^{N}/_{2}$ alcoholic potash.

Time, in hours.	Saponification of Bromo-glyceride. Per cent Bromine.	Saponification of Bromide from acids. Per cent Bromine.
Till ppt. dissolves.	44.48	.....
.5	47.04	50.56
1.	50.40	54.70
2.	.....	57.12
4.	54.08	60.16
6.	55.04	61.12

Using Method A., the bromo-glyceride gave 55.20 per cent of bromine, and the bromide from the acids 60.96 per cent. Thus Method A. appears to be quite as accurate as, and much more rapid than, Method B., and was the one used in all determinations.

Bromine Content of the Bromo - Glycerides. The bromine content of the bromo-glycerides obtained from several samples of linseed oil was determined, with the following results:-

	Per cent Bromine.
Sample A. - Raw.	56.00
B. "	57.28
C. "	56.64
D. - Boiled.	52.48
E. "	53.29

It appears, therefore, that the precipitate has a more or less definite composition in the case of raw linseed oils, but/

but that in the case of the boiled oils the bromine content is lower and not so constant, no doubt due to the presence in the oil of small amounts of lead and manganese driers which form insoluble compounds with the bromine derivatives of lower fatty acids.

The formula suggested by Hehner & Mitchell is the one which most nearly approximates in bromine content to the precipitates, and the suggestion of these authors that the low bromine content is due to the fact that the linolenic acid is present, not as the pure glyceride but as a mixed glyceride with oleic acid, appears to be quite a feasible one. On the other hand, the result of an examination of the precipitates obtained by brominating in chloroform - ether solution in place of ether, (P. 17), seems to prove that the combination of the linolenic radicle with some other radicle is not so strong as to pre - suppose the presence of a mixed glyceride without very strong proofs, as the bromine content varies according to the proportion of ether to chloroform in the solvent mixture.

Sample 1.

c.c. of ether.	c.c. of chloroform.	Per cent of Bromide.	Per cent of Bromine.
20	20	25.08	59.23
30	10	28.73	55.98
35	5	35.63	54.28
40	0	35.80	53.62

Sample 2.

c. c. of ether.	c.c. of chlofoform.	Per cent of Bromide.	Per cent of Bromine.
20	20	26.50	59.20
30	10	32.45	56.64
40	0	56.64	56.00

Melting Point of the Bromo - glycerides. Previous investigators more or less agree that the melting point of the bromo - glycerides is about 140° C. Hehner & Mitchell state 143.5°- 144°C., Sutcliffe, 140°- 143°C. When determining the melting point of the precipitates obtained from various samples of oil, it was noticed that the true point could not be determined exactly to 1° C., but that there appeared to be a gradual softening from 140° C. upwards. Again, this softening point was the same in the case of all the precipitates obtained by bromination in ether - chloroform solutions, irrespective of the proportions of the two substances used in the mixture and consequent difference in the bromine content. Still further, the fused substance in the melting point tube did not re - crystallize on cooling, but remained clear and glassy. Thus it appeared doubtful if this was a true melting point or a point of decomposition. That the latter was actually the case, was proved by the fact that after heating at 145° C. till the whole mass became fused and the melt was subsequently cooled, a large proportion of the melt was soluble in ether, whereas the original solid was practically insoluble in that solvent.

A quantitative estimation of the ether soluble portion of the fused mass was made. 1 gram of glycerides was heated to 145° C. and allowed to remain at that temperature for half an hour, then cooled and weighed. There was no loss in weight, thus showing that any decomposition was a simple splitting up. The fused mass was then allowed to stand in contact with ether for several days, the extract decanted, and the undissolved solid re-extracted. The ethereal extract was a dark oily/

oily substance, weighing .413 grams, and containing 46.1 per cent of bromine. The original precipitate contained 56.0 per cent of bromine, and theory required that the remaining .587 grams should contain .3696 grams of bromine or 62.9 per cent. The figure actually obtained was 61.63 per cent. It is thus apparent that the so called melting point is a point of decomposition, the bromide splitting up into two compounds one of which closely corresponds in bromine content to the hexabromide or hexa-bromo-glyceride. As regards the ether soluble body containing 46.1 per cent of bromine, the author has not had the time available for a detailed examination as to its nature. On saponification it yielded the alkali salt of an acid whose neutralisation value was found to be 175.3 which lies in value between that required for dihydroxystearic acid and that for trihydroxystearic acid. The extract may possibly consist of a mixture of the bromo-glycerides of oleic and linoleic acids, but no definite statements can be brought forward to prove this.

Bromine Content of the Bromo - Acids. The bromides obtained by direct bromination of the acids themselves were found to vary according to the sample of oil under examination and also according as acetic acid was present or absent in the solution during bromination.

Linseed Oils.	Percent Bromine.	
	In presence of Acetic Acid.	In absence of Acetic Acid.
Sample A. (Raw Baltic).	63.16	61.44
B. " "	63.04	61.12
C. (Raw Plate).	61.92	60.48
D. (Boiled).	62.24	61.12
E. "	62.98	61.52
Soya Bean.	60.19	58.13
Mixture of 1 Linoleic Acid. 2 Linolenic Acid.	62.40	60.80

These figures taken in conjunction with the tables on pp. 23 - 24 prove that the precipitates consist essentially of linolenic hexa-bromide with varying proportions of linoleic tetra-bromide. Naturally the actual proportion would vary according to the proportions of each in the mixed fatty acids from the oil under examination, hence the fact that the percentage is not a constant one, and also that there is no definite relationship between the percentage of precipitate and the iodine value.

It was also mentioned on p.24 that results were about 16 per cent lower when acetic acid was present during bromination, but that the precipitates were purer, hence it was advisable to use it. The correctness of this statement is proved by an examination of the table of results on the preceding page. This was further proved as follows:-
In a solution containing 2 per cent of linolenic acid and 1 per cent of linoleic acid, the precipitate weighed .326 gram and contained 60.8 per cent of bromine, when precipitated in absence of acetic acid. The same volume of solution containing 10 per cent by volume of the acid yielded a precipitate which weighed .283 gram and contained 62.4 per cent of bromine. 60.8 per cent of bromine corresponds to 25.33 per cent of linoleic tetra-bromide and 74.76 per cent of linolenic hexa-bromide, while 62.4 per cent of bromine corresponds to 9.31 per cent of linoleic tetra-bromide, and 90.69 per cent of linolenic hexa-bromide. Thus the loss in weight is compensated by a corresponding gain in purity.

The table also shows that Baltic linseed oils contain a greater relative proportion of linolenic acid to linoleic/

linoleic acid than the Plate linseed oils, as the precipitates more nearly approximate to linolenic hexa-bromide, also that soya bean oil contains a low relative proportion of linolenic acid to linoleic acid.

With regard to the bromine content of the precipitates obtained from the marine animal oils these also vary and correspond to mixtures of linolenic hexa-bromide and the compound $C_{17}H_{26}O_2Br_8$, (which theoretically has a bromine content of 70.96 per cent). Hehner & Mitchell give a figure for the bromides from cod liver oil fatty acids of 62.91 per cent. This is much lower than found in the present investigation.

Nature of Oil.	Percentage Bromine.	
	In presence of Acetic Acid.	In absence of Acetic Acid.
Pale seal.	67.65	66.83
Shark liver.	67.60	66.80
Cod liver.	68.87	67.84
Whale.	67.21	66.32

Melting Point of Bromo - Acids. The melting point of the bromides from the acids was very sharply defined as compared with the fusing point of the bromo-glycerides, complete fusion taking place within limits of .5°C. As is to be expected, the melting points varied slightly owing to the variation in the composition of the precipitates. The melting points ranged from 179°C. to 181°C. In all cases the fused bromides re - crystallized on cooling.

SUMMARY.

Briefly, the results achieved by the foregoing investigation may be summarised as follows:-

1. Of the published methods, none give absolutely concordant results.

2. No means have been found to so far modify those methods in which the glycerides are directly brominated, as to give concordant results.

3. A reliable method is to be found in the modification of Bull &. Johannsen's method as described on pp. 18 - 19, with the method of washing recommended on p. 21. This method is equally applicable to an examination of vegetable or marine animal oils.

4. The nature of the precipitated bromo - glycerides is indefinite and the so called melting point is actually a decomposition point, at which the product splits up into a pure hexa-bromide or hexa-bromo-glyceride, and a compound or compounds of lower bromine content, the actual nature of which requires further investigation.

5. The bromides, obtained by bromination of the fatty acids of the vegetable oils, consist essentially of a mixture of linoleic tetra-bromide and linolenic hexa-bromide, in composition more or less closely approximating to the latter substance. In the case of the marine animal oils the precipitates consist of a mixture of the hexa-bromide and the compound $C_{17}H_{26}O_2Br_8$.

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